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## A FURTHER ANALYSIS OF THE HEREDITARY TRANSMISSION OF DEGENERACY AND DEFORMITIES BY THE DESCENDANTS OF ALCOHOLIZED MAMMALS

CHARLES R. STOCKARD AND GEORGE PAPANICOLAOU

DEPARTMENT OF ANATOMY, CORNELL UNIVERSITY MEDICAL SCHOOL,  
NEW YORK CITY

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### INTRODUCTION

A LITTLE more than two years ago the senior author (Stockard, '13) recorded in this journal experiments which had then been running for three years and seemed to show a definite injury of the germ cells by treating mammals with the fumes of alcohol. This injury of the male germ cells is of such a nature that an alcoholized male guinea pig almost invariably begets defective offspring even when mated with a vigorous normal female. At that time it was also shown that  $F_1$  animals, the offspring of treated parents, though themselves not treated,

had the power to transmit the defective condition to their young, and such  $F_2$  young were equally if not more defective than the immediate offspring of the treated animals.

In 1914 in a short abstract Stockard showed further that the offspring from  $F_2$  individuals were apparently more defective than their parents and were often grossly deformed. One case was recorded of the occurrence of a litter of two  $F_3$  animals, both of which were extremely weak and neurotic, showing a condition suggesting paralysis agitans, and further than this the two animals were typical anophthalmic monsters. The eyes were completely absent, no optic nerve or optic chiasma or visible optic tracts along the tuber cinereum could be found on a careful gross examination of the brain. The two animals were produced by parents ( $F_2$ ) that had never been treated with alcohol, the four grandparents ( $F_1$ ) had also not been treated, while the *three great grandfathers* had been alcoholized and the three great grandmothers were normal untreated individuals.

Defective eyes and absence of one eye or both eyes have been frequently met with in the experiment, as well as the peculiar nervous condition, and these symptoms are to be considered indicative of the injury or change induced in the male germ cells by the experimental treatment, which in the above case was transmitted through three generations. No question could remain as to the action on the germ cells, as only male ancestors had been treated; every female of the line was an untreated animal.

This abstract called attention to the fact that there was a tendency for the results to differ in subsequent generations from treated males as compared with the descendants of treated females—not enough data were then present to offer any explanation of these differences and a consideration of them will be undertaken in the present paper.

At that stage of the experiment it was also difficult to offer an exact analysis of the mode of transmission of

the defects and the type of injury induced by the alcohol treatment, since the total numbers were not large and the  $F_2$  animals had only a few matings, while further generations had not become available for breeding.

The same experiments have now been continued for more than five years and a number of animals have been used, over 700, which cover the behavior of four generations and supply data of sufficient extent to allow a more thorough analytical consideration of the heredity problem concerned.

Experiments of this nature on mammals are fraught with many difficulties, slowness of breeding, small size of litters, difficulty of handling, etc. Yet such material offers one very great advantage in that the quality of the offspring and generations studied is of such a complex that one is enabled to detect indications of rather slight injuries or changes in the material carriers of heredity which would not become evident on lower forms with less diversity in their methods of behavior and structural appearance. In other words, we take it that such conditions as are spoken of as racial degeneracy in man and mammals are often very difficult or even at times impossible to detect in lower forms.

These conditions are for many reasons thought to be inherited. If so their inheritance must be due to a pathological condition of the material carriers of heredity, the chromosomes, or what not, since they are not normal states and, like diseases, are constantly arising in normal families on account of one or another form of intoxication. Is it possible then to produce such a racial degeneracy artificially by treating only one generation of the animals and by so doing observe a pathological behavior of the carriers of heredity? Arguing from analogy there must be pathological heredity due to diseased or altered chromosomes in the germ cells just as truly as there is a known pathological behavior of every other organ and tissue of the animal body.

It becomes then a problem to study the possible meth-

ods of modifying the chromosomes or carriers of the inherited qualities of organisms in order to further analyze their normal physiological behavior; in the same way that experimental embryology has been able to supply so many valuable clues to the normal processes of development.

In the following pages we believe the facts indicate that individual guinea pigs are now living in this experiment that have had the carriers of hereditary qualities, the chromatin, of their germ cells injured for a longer time than four years. And during this time they have given rise to offspring of more or less degenerate or deformed type, and in some cases these offspring have passed this modified chromatin on through three generations, all of which contain pathological chromatin and show somatic defects and deformities as an index of their tainted chromatic ancestry. Modified chromatin has been living in the experiment for more than four years in five different generations of animals as a result of the treatment on the one original,  $P_1$ , parent generation.

We have tried to regulate every controllable source of error and there can be no doubt that the conditions are brought about in the way described. Could the degeneracy which is so pronounced have previously existed in the stock? This question has been controlled in the first place by the use of two entirely different stocks from different sources and obtained one and one half years apart, the first in the fall of 1910 and the other in the early winter of 1912. The responses of the two stocks to the experimental treatment have been identical. As a second method of control every animal has been tested by one or more normal matings before being introduced into the experiment, and only those giving normally strong offspring have been used. A further crucial control is the constant mating of normal untreated animals from both stocks under identical cage conditions with the experimental individuals. These animals continue to breed normally until very old, when they gradually become

sterile. But none have ever given rise to a defective or deformed individual, and the rate of mortality of the young indicates the average healthy condition found in normal guinea-pig breeding. There is a striking contrast between the records of these normal young and the mortality record, the frequency of easily recognized nervous symptoms of degeneracy, and the prevalence of gross deformities in the experimental races.

The external as well as internal factors are to be considered not only in individual or embryonic development, but also in heredity. And the present experiments now demonstrate for mammals that either the spermatozoon or the ovum may be experimentally injured or modified in such a manner as not only to give rise to (abnormal) subnormal development in the resulting embryo, but the effects of the injury may be transmitted from generation to generation, until an affected line actually fades out through degeneracy and sterility as a result of the transmitted condition.

#### MATERIAL AND METHODS

The animals used in the experiments have been ordinary vigorous guinea pigs of large size, particular care being taken to select animals less than one year old to begin with and good breeders.

At the beginning of the experiments alcohol was given along with the food, but the animals ate less and the food usually disagreed with them. It was then administered in diluted form by a stomach tube; this method was even more unsuccessful, disturbing digestion and seeming to upset the animals considerably. It is certain that alcohol given to animals through the stomach deranges their appetite and digestion to such an extent that the experimenter is unable to determine whether the resulting effects are due to the alcohol, as such, or to the generally deranged metabolism of the animal. When given in drinking water they take little or none of the water and the treatment is insufficient. For these reasons an inha-

lation method of treatment was resorted to early in the study, and, as far as experience goes, it has no serious disadvantages and does not complicate the conditions of the experiment.

This method may be merely described in brief for the convenience of the reader, since it has been fully recorded with illustrations of the fume tanks in previous publications. A fume tank of copper is made of sufficient size to supply breathing space for four or five guinea pigs at one time. The tank has four outlets, so that a definite amount of fumes may be passed through in a given time and the ventilation controlled. In this way each animal could be given a definite measured dose. The individuals, however, differ so much in their resistance to the treatment that it has been found better to treat all to about the same degree of intoxication. Such a physiological index is more reliable, since every animal may be affected to the same degree each day. For this purpose the animals are placed in the fume tank on a wire screen, and absorbent cotton soaked with alcohol is placed beneath the screen, so that they inhale the alcohol fumes arising from the cotton to saturate the atmosphere of the tank.

Ether was given in a similar manner. The animals are much more readily overcome by these fumes and must be carefully watched while inhaling even the most dilute doses.

To avoid handling the females during pregnancy, special treating cages are devised. An ordinary box-run with a covered nest in which the animal lives is connected by a drop-door with a metal-lined tank, having a similar screen arrangement, etc., to that of the general treatment tank. The pregnant animal may be driven daily into the tank and thus treated with alcohol fumes throughout her pregnancy without being handled in any way that might disturb the developing fetus.

Particular care is necessary in mating the animals in such an experiment, as the females are often slow to con-

ceive and some of the  $F_2$  and  $F_3$  individuals of both sexes are not very prolific and in many cases are almost or quite sterile. Each female is kept in a separate run and the male is placed in with her just before the time of the expected heat period, ovulation, and he remains in her cage for from two to three weeks so as to be present at the second ovulation, provided the female was not made pregnant by the first mating. The ovarian cycle of the guinea pig as worked out by L. Loeb seems to correspond closely to what is found in mating experiences.

After mating, the male is removed from the cage and the female remains alone until the young are born. These are left with the mother for about fifteen days, then separated, and the female mated again. In this way the normal females may sometimes give as many as four litters per year, but the experimental animals breed much slower and it is difficult to get even three litters per year.

#### DIRECT EFFECTS OF THE ALCOHOL TREATMENT ON THE ANIMALS

Several of the guinea pigs have now been treated with the fumes of alcohol almost to the point of intoxication for six days per week for a period of five years. This is a considerable space in the life of a guinea pig, which probably would not often extend beyond six or seven years.

The animals are affected by the alcohol fumes in various ways; some of them are stupefied and become drowsy, while others become stimulated and excited and sometimes even vicious, constantly fighting and biting at the other animals in the fume tank. The fumes inhaled into the lungs pass directly into the circulation, so that the animals show signs of intoxication very soon after being put into the tank, yet the intake of alcohol is so gradual that they may remain for one hour or more without becoming totally anesthetized.

The mucosa of the respiratory tract is considerably

irritated during the early stages of the treatment, but develops a resistance so that later little effect can be noticed. The cornea of the eye is greatly irritated, often becoming milky white and opaque during the first few months. In some cases this later clears and the animal is again able to see, though some of the animals treated for several years have remained entirely blind. The general condition of the animals under the fume treatment is very good. They all continue to grow if the treatment is begun before reaching their full size, and become fat and vigorous, taking plenty of food and behaving in a typically normal manner.

Some of the treated animals have died and others have been killed at different times during the progress of the experiment and their organs and tissues examined carefully and then studied microscopically. All have seemed practically normal. Tissues from several animals treated as long as three years have been examined and the heart, stomach, lungs, kidneys, and other organs present no noticeable conditions that might not be found in normal individuals. Alcoholized animals are usually fat, but there is no fatty accumulation in the parenchyma of any of the organs.

Several of the animals, both males and females, have been partially castrated during the experiments and the ovaries and testes have been found to be in a healthy condition, though certain possible changes may be present which are now being closely studied cytologically and experimentally.

The treated animals are, therefore, little changed or injured so far as their behavior and structure goes. Nevertheless, the effects of the treatment are most emphatically shown by the type of offspring to which the alcoholized individuals give rise, whether they be mated together or with normal individuals. The further significance of the nature of the effects is indicated by the quality of the subsequent generations descended from such an ancestry.

INFLUENCE OF THE TREATMENT ON THE DESCENDANTS OF  
ALCOHOLIZED ANIMALS

It may be well in the first place to consider the results of the experiments from a general standpoint and then to undertake an analysis of the reactions and conditions presented in the several generations and from the several lineal combinations. The records of the matings of the alcoholized animals in various pairs, the control or normal matings, and the matings of the  $F_1$  and  $F_2$  generations, the children and grandchildren of the alcoholized individuals are summarized in the general Table I. This table gives a record of all the matings of the kinds indicated up to July 1, 1915. A similar table was published two years ago, when the number of animals considered was much smaller and the actual indications from the results were less certain than now. On comparing this table with the former one, however, it will be seen that the continuation of the experiments has fully substantiated the results as previously recorded. The table now shows the records of 571 matings which produced 682 full-term young and 189 early abortions or negative results. These numbers are now of considerable magnitude in spite of the fact that the experiment is conducted on mammals which produce only small litters and breed slowly as compared with lower animal forms.

In the first horizontal line the record of pairing alcoholized male guinea pigs with normal females is given. This combination could only produce defective or subnormal young as a result of the injured male germ cells, since the ova are normal and develop in a normal untreated mother. This then is the definite test of the influence of the alcohol treatment on the germ cells.

Ninety such matings have in 37 cases given negative results; that is, failures to conceive, or early abortions. Thus 41 per cent. of the matings of such males were non-productive, while less than 25 per cent. of normal matings under the same breeding conditions failed to produce full-term litters. Ten stillborn litters, each consisting of

two young, twenty stillborn young, resulted from the 90 matings. While the 90 control matings gave only two stillborn litters, and in both cases these were unusually large litters of four individuals each, and they were probably dead on account of the fact that the mother could not give normal birth to so many offspring. The stillborn litters by the alcoholized fathers were all ordinary-size litters of two young. Thus, while 11 per cent. of the matings of alcoholized males resulted in stillborn litters, only 2 per cent. stillborn litters occurred from normal matings. Forty-three living litters were produced or a little less than 48 per cent. of the matings gave full-term living young, while 73 per cent. of the normal matings give living litters of young.

The 43 litters from alcoholic fathers contained in all 82 young, and 35, or almost 43 per cent., of these died soon after birth, while 66 similar litters from the control lost only 19 young, or 16 per cent., out of 118 individuals. Finally, then, from the 90 matings of alcoholic males with normal mates only 43 full-term litters resulted, consisting in all of 102 young; 55 of these, or 54 per cent., died at birth or soon after, and only 47 individuals, or 46 per cent., survived. Only about half as good record as the 78.5 per cent. surviving young from the matings of normal animals. Almost all of the offspring were very excitable, nervous animals and three of them showed gross deformities of the eyes, while no such conditions were found among any of the offspring of normal animals bred under identical conditions.

These records leave no doubt that the alcoholized male guinea pig is injured in such a way as to induce a decidedly bad effect upon the quality and mortality of his offspring when compared with the records from normal animals.

The second horizontal line of Table I shows the results obtained when alcoholized female guinea pigs are paired with normal males. In this case there is a double chance to injure the offspring. First through the influence of

the treatment on the oocytes or the unfertilized ovarian egg, a direct effect on the germ cells comparable to the injury of the germ cells in the case of the treated males considered above. While in the second place, the developing embryo in the uterus of an alcoholized female may be directly affected by the strange substances contained in the blood and body fluids of the mother. Thus a defective individual may be produced as a result of development in an unfavorable environment or as a result of being derived from an injured or defective egg cell.

Thirty-three matings of alcoholized females with normal males have in seven cases, 21 per cent., given negative results or early abortions; this compares very favorably with the records of the control animals. Four stillborn litters consisting of three individuals each were produced. This is a record of 12 per cent. stillborn litters against only 2 per cent. from normal matings. The alcoholized females gave birth to 22 living litters containing 44 young, and 23, or 52 per cent., of these died, only 48 per cent. surviving against 84 per cent. survivals among the young of similar control litters. The records of the matings of alcoholized females compare very unfavorably with the record of the control matings. Yet the behavior of the alcoholized females is very little, if any, worse than the records shown by the alcoholized males in spite of the double chance the female has to injure her young.

The third horizontal line of the table indicates the results obtained when alcoholized males are paired with alcoholized females. Here there is every chance for the treatment to show its effect. The percentage of early abortions or negative results is very high, about 50 per cent. more than double that of the control matings. Ten per cent. of the matings produced stillborn litters each consisting of two young. Only 17 living litters were born out of 41 matings, about 41 per cent., against 73 per cent. living litters from 90 control matings. The 17 living lit-

ters contained only 26 young, and 12 of these, or 46 per cent., died soon after birth, while but 16 per cent., or one third as many, of the control offspring died out of a total of 118 individuals. The data from the double alcoholic matings is, therefore, extremely bad in the light of normal matings from the same animal stocks bred under exactly the same cage and food conditions.

The fourth horizontal line summarizes the records of all the matings of directly alcoholized animals. In all 164 such matings have been made; 64 of these, or almost 40 per cent., gave negative results or early abortions. Eighteen stillborn litters occurred, consisting of 40 individuals against only two questionable stillborn litters from 90 control matings. Eighty-two, or only 61 per cent., living litters were born, consisting of 152 individuals, 82, or 54 per cent., of which survived and 70, or 46 per cent., died soon after birth; in all 110 full-term young died, while only 82, or 42 per cent., of the total 192 full-term young resulting from the 164 alcoholic matings survived. On the other hand, out of a total of 126 full-term young from only 90 control matings, 99, or 78.5 per cent., survived. The control matings were far more prolific than those of the alcoholized animals and the condition of the young as indicated by the mortality record was far superior to that of the alcoholic offspring.

The fifth line records the outcome of 90 control matings which have been scattered through the entire progress of the experiment under exactly the same conditions and from the same animal stocks as the experimental matings. Eighty-four per cent. of the young in the 66 living litters resulting from the matings of normal animals have survived and all are strong, healthy individuals; in not one instance do they show an indication of nervous degeneracy or any type of recognizable structural deformity, while such degeneracy as well as deformities are extremely prevalent among the offspring and descendants of the alcoholized animals. One other point to be mentioned in considering the records of the

TABLE I  
EFFECTS OF ALCOHOL ON THE DESCENDANTS OF TREATED ANIMALS

Condition of the Animals	Number of Matings	Negative Result of Early Abortion	Stillborn Litters	Number of Stillborn Young	Living Litters	Young Dying Soon After Birth	Total Dead	Surviving Young
Alcoholic ♂ × norm. ♀	90	37	10	20	43	35, 1 c.e.	55	47, 2 c.e.
Norm. ♂ × alcoholic ♀	33	7	4	12	22	23	35	21
Alcoholic ♂ × alcoholic ♀	41	20	4	8	17	12	20	14
Summary	164	64	18	40	82	70	110	82
Control norm. ♂ × norm. ♀	90	22	2	8	66	19	27	99
♀ treated during pregnancy	4	0	0	0	4	1	1	7
Second generation × norm.	46	10	3	8, 6 c.e.	33	29, 2 par.	37	25, 3 c.e.
Second gener. × alcoholic	53	16	8	17, 1 d.e.	29	22, 3 d.e.	39	28
Second gener. × second gener.	95	29	7	16	59	43, 2 par., 6 d.e.	59	52, 3 d.e., 1 one e., 1 eyeless
Third gener. × third gener.	48	20	7	14, 1 d. legs	21	19, 1 par., 6 d.e., 2 eyeless	33	13
Third gener. × second gener.	33	15	4	8	14	16, 1 par., 1 c.e.	24	7
Third gener. × normal	17	3	4	8	10	5	13	7
Third gener. × alcoholic	3	1	0	0	2	2	2	1
Second, third gener. × second, third gener.	18	9	2	6	7	6	12	4

control matings is the fact that from 90 matings only two stillborn litters were produced and, as mentioned above, both of these litters were of so large a size that the mothers seemed unable to successfully deliver them and one of the mothers failed to recover from the process and died a few days later. These two cases make the control records appear worse than they actually should, but in spite of this the control matings have given data equally as good as those generally obtained by careful breeding experiments with vigorous normal stocks. The stock in these experiments is unquestionably good, as the control matings very readily show.

Four normal females were mated and then treated with alcohol throughout their periods of pregnancy and, as the sixth horizontal line of the table indicates, such a treatment was not at all injurious in these particular cases. It actually happened that some of these young were unusually vigorous. The numbers are very small, but this is a direct test, and if such a treatment were really decidedly effective in its action on the embryo or fetus *in utero* these eight young animals should have at least shown some response. It is very possible that after the treatment has been continued for a long time, a year or more, that the mother then presents a uterine environment unfavorable for normal development, since the offspring of such individuals are almost always subnormal. In these cases, however, the inferior quality of the offspring may be due to the action of the alcoholic treatment on the ovarian germ cells rather than the direct environmental effect on the developing embryo or fetus, there is no way at such a stage to separate the two possible effects.

The next three horizontal lines, seventh, eighth and ninth, give the data resulting from the matings in various combinations of the  $F_1$  animals, that is, offspring from alcoholic parentage, but which are not themselves treated with alcohol. The records of these non-treated  $F_1$  individuals are most instructive for an understanding of the actual influences of the alcoholic treatments.

When such  $F_1$  animals are paired with normal individuals the seventh line shows that almost 22 per cent. of the matings failed, which is not a bad record. The proportion of stillborn litters, however, from the  $F_1$  by normal combination was three times as great as from normal matings and 75 per cent. of the stillborn young produced showed gross defects of the eyes, having opaque lenses or typical cataract conditions, while not one of 126 young from normal matings has shown this or any other noticeably abnormal structure. Thirty-three living litters were produced containing in all 54 individuals, 29, or 54 per cent., of which died soon after birth, while 25 survived. Two of those dying soon after birth were paralyzed and unable to walk, while three of the 25 survivors have defective opaque eyes, cases similar to that illustrated by Fig. 1, and many show different nervous symptoms. Thus of 62 full-term young produced by  $F_1$  animals with normal mates, only 25, or 40 per cent., survived for more than a short time after birth, and 12 per cent. of these have gross defects and more than half of them are nervous, excitable individuals, which when mated with normal animals or in any other combination always give very poor quality offspring, if any at all.

The eighth line shows the records of 53 matings between  $F_1$  animals and alcoholics. This combination again gives data comparing most unfavorably with the control and in some ways even worse than the records of matings between two alcoholic animals. Fifteen per cent. of such matings produced stillborn litters! Only one combination gives a worse record of stillborn that is, matings among  $F_2$  animals. Almost half of the young in the living litters died and here again some were deformed. Deformities are strikingly more abundant among the offspring from  $F_1$  and  $F_2$  parents than from the directly alcoholized animals.

The record of 95 *inter se* matings of  $F_1$  animals is shown in the ninth line. Thirty per cent. of such matings gave negative results or early abortions, over 7 per

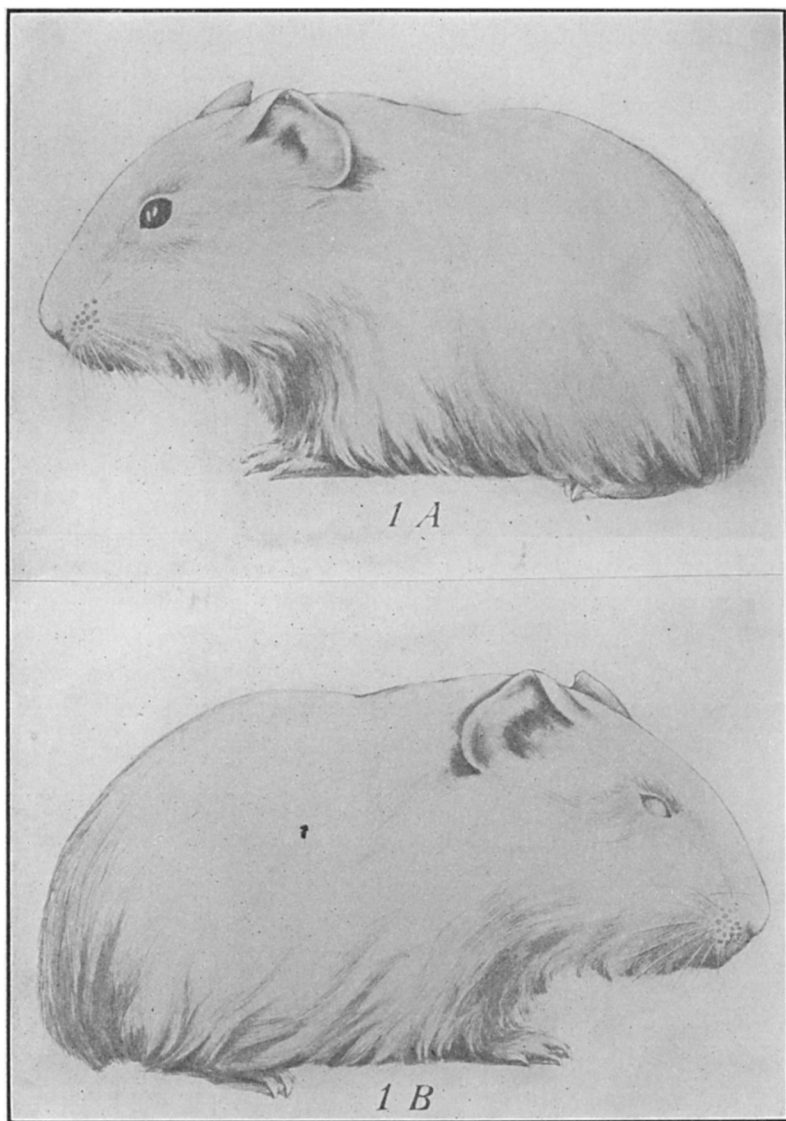


FIG. 1, A and B. 271 F<sub>2</sub> ♀ (one in litter) (AA)(AN). Both paternal grandparents and the maternal grandfather were alcoholic, no inbreeding. The right eye is smaller than the left and has been entirely opaque since birth. This animal, almost two years old and vigorous, is entirely sterile.

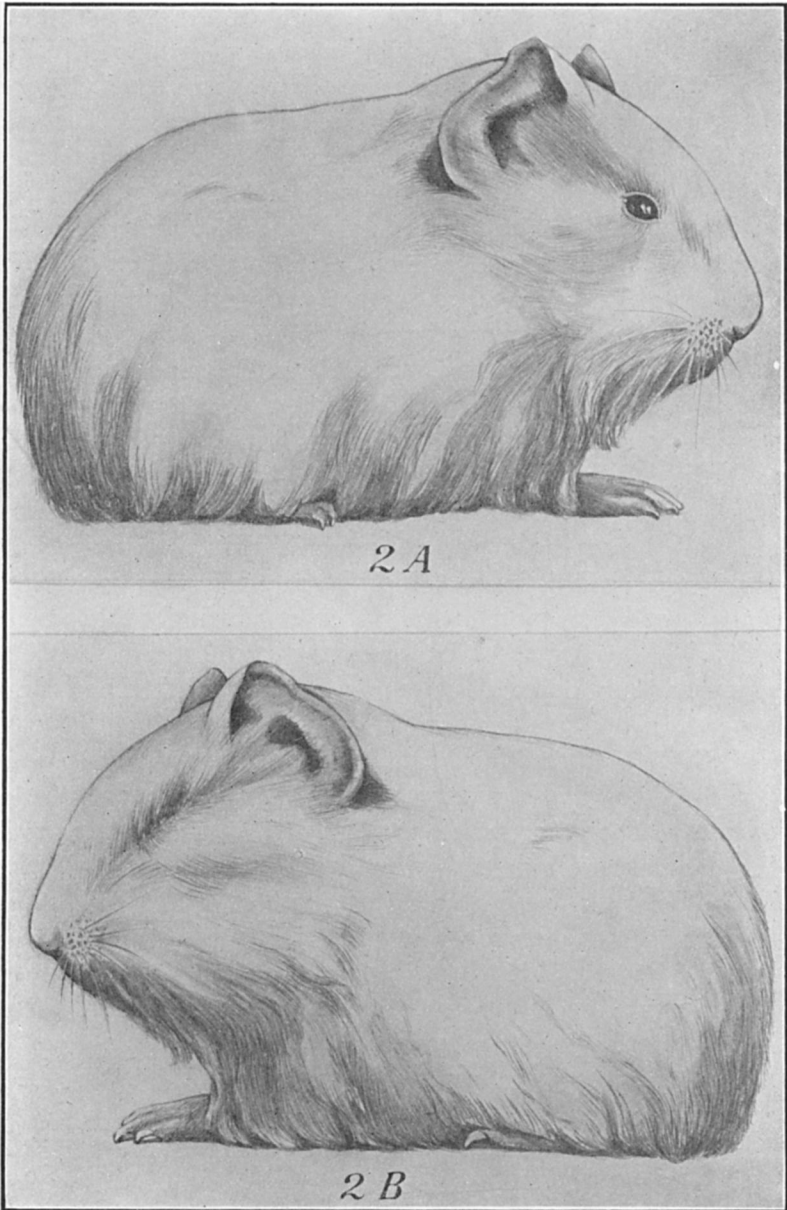


FIG. 2, A and B. 307  $F_2$  ♀ (one in litter). Inbred, from brother and sister offspring of an alcoholic male (AN) (AN). The eye of one side normal, the other eye ball apparently absent on living examination. A typical monster monophthalmicum asymmetricum. This animal now 21 months old is completely sterile.

cent. stillborn litters and 62 per cent. living litters. Little less than half of the living young died soon after birth, in all 43, nine of which, or more than one in five, 21 per cent., were paralyzed or deformed; the figures in Plates I and II illustrate the paralytic conditions. Fifty-two of the offspring survived, three with deformed eyes, one with one eyeball completely absent, monster monophthalmicum asymmetricum (Fig. 2, 307 ♀), and almost all of the 52 are very nervous, excitable animals which when bred give rise to deformed or highly degenerate offspring.

The offspring from the  $F_1$  animals mated in any combination are generally far below the normal in power to survive and in quality of structure. When compared with the offspring from directly alcoholized parents, the offspring from the  $F_1$  combinations show an equally bad mortality record and a very much higher proportion of paralyzed and deformed individuals. The 95 matings *inter se* of  $F_1$  animals demonstrate conclusively that such individuals carry defective or abnormal germ cells which give rise to defective developmental products. These degenerate  $F_2$  offspring owe their subnormal condition to the effects of the action of the alcohol treatment upon the germ cells of their grandparents which have been transmitted to them through their parents. In other words, the carriers of hereditary qualities have been modified in the first parental generation, and the effects of this modification are expressed in their offspring  $F_1$ , and also in their grandchildren, the  $F_2$  generation.

The next line of the table, the tenth, indicates further how the effects of the original modification are transmitted to the great grandchildren or through three generations since the injury. Forty-eight *inter se* matings of  $F_2$  animals gave the results here shown. Almost 42 per cent. of the matings gave negative results or early abortions, the poorest record in this respect shown in the entire table. About 15 per cent. of such matings gave stillborn litters, 7 in 48 matings, which is remarkably high when compared with any of the above combinations.

The hind legs of one of the stillborn young were deformed in the peculiar manner illustrated in Figs. 4 and 5.

Twenty-one living litters were produced, containing in all 32 young; 19 of these, almost 60 per cent., died soon



FIG. 3. F<sub>3</sub>. Two in litter, both same condition, three normal great-grandmothers and three alcoholic great-grandfathers. The parents were single first cousins. Both animals completely eyeless, also with paralysis agitans, one died the second and the other the third day after birth, typical anophthalmia. One brain no indication of optic nerve, the other slight processes.

after birth, and only 13 survived. One of the 19 that died was paralyzed and unable to stand, while 8 of them, a strikingly high proportion, were grossly deformed. Six

had one or both eyes deformed (Figs. 1 and 2), and two were anophthalmic monsters, being completely without eyeballs, optic nerves, optic chiasma or any gross signs of optic tracts (Fig. 3). The brains are now being studied in sections. Figs. 1 to 3 illustrate animals showing the different eye conditions—asymmetrical eyes, monstrem monophthalmicum, and anophthalmic monsters. Figs. 5 and 6 of Plate III illustrate the brains of a normal and an anophthalmic specimen for a comparison of the condition of the optic nerves, etc.

Forty-six full-term young were produced by the  $F_2$  matings, but only 13 of these, or just 28 per cent., were able to survive, while about three times this proportion, or 78.5 per cent. of the full-term young from control matings, survived as vigorous healthy individuals. The 13 living  $F_3$  animals are all rather weak and degenerate and almost completely sterile according to a considerable number of careful matings with strong, fertile guinea pigs. The alcoholic race seems at this stage of the experiment about to fade out in the fourth generation, while normal control lines from the same original stocks have passed far beyond this generation, continuing to breed normally and showing no signs of degeneracy, and never in any case giving rise to a grossly deformed animal.

The eleventh line indicates again the very decided effects transmitted by the descendants of animals which had suffered a modification of their germ plasm by the alcoholization of their tissues. In 33 cases  $F_1$  and  $F_2$  animals were paired together. Fifteen of these matings gave negative results or early abortions, while about 12 per cent. of the matings resulted in stillborn litters of two young each. Only 14 living litters were produced by the 33 matings; these contained in all 23 young, only 7 of which survived. Thus from a total of 31 full-term young only 7, or about 22 per cent., were capable of surviving. All of these young animals are nervous and weak and several offspring from these combinations were deformed.

When  $F_2$  animals are mated with normal individuals

the results are very little if any improved over the two above combinations. Seventeen such matings gave only three failures or early abortions, but a high proportion, 23 per cent., of stillborn litters arose, while 10 living litters, consisting of only 12 individuals, were born. In all 20 full-term young were born and only about one in three of them survived. In this experiment, although one mate was a normal animal, the  $F_2$  mate carried germ cells of so inferior a quality that the output of the combination, admitting the numbers are small, leaves no doubt of the transmission, *through three generations*, of defective conditions induced by alcoholizing the great grandparents of the offspring on only one side of the family, or in only one of the parental lines.

The last line of the table gives the records of mixed combinations of  $F_1$  and  $F_2$  individuals, and here the data are closely similar to those obtained from other combinations of these animals; only about 25 per cent. of the full-term young born are capable of surviving, while 78.5 per cent. of the control young are living.

Briefly, then, 571 matings tabulated in Table I, the records to July 1, 1915, have given rise to 682 full-term young, as well as a large number of premature abortions. A careful study of all these young animals extending over a period of five years has afforded data which convincingly show that the treatment of either the male or the female guinea pig with fumes of alcohol affects the quality of the offspring to which these animals give rise even when paired with normal mates. And further, the changed quality of the offspring is subsequently transmitted through succeeding generations with even more severe marks of degeneration and deformity than those exhibited by the offspring of the directly treated animals.

Other combinations and back crosses are now in progress which are fully in line with the above, but which have not yet afforded sufficient analytical data to record.

The defects caused by the alcohol treatment seem to be largely confined to the central nervous system and organs

of special sense. Paralysis agitans is very common among the  $F_1$ ,  $F_2$  and  $F_3$  animals. Paralyzed limbs are often observed, the animals being unable to stand or walk (Plates I and II). The eye is also a peculiarly sensitive indicator and presents in the various descendants of alcoholized individuals all degrees of degeneration—

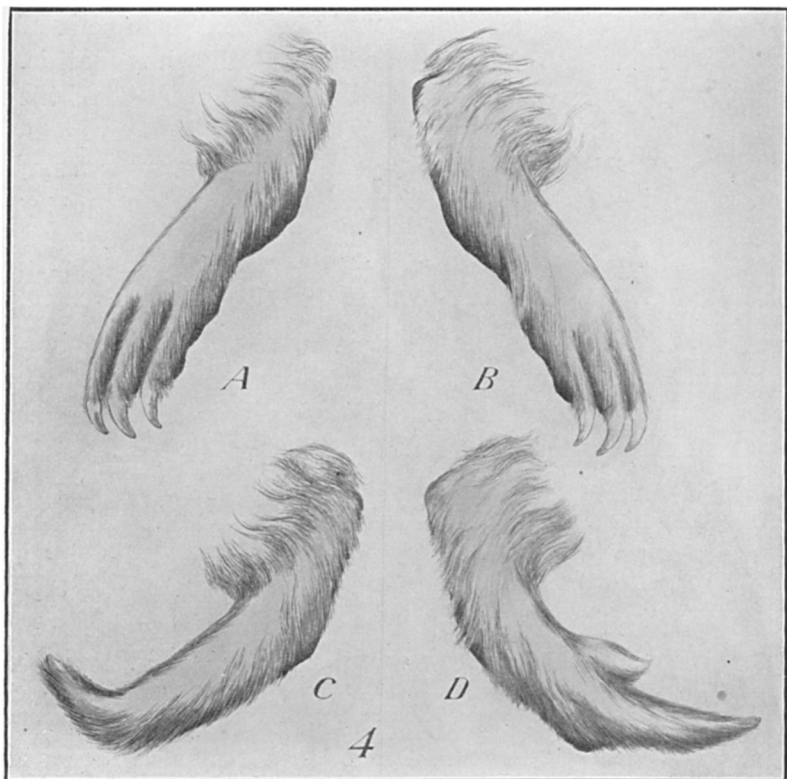


FIG. 4. Hind feet of No. 488  $F_2$ , s ♀. All great-grandparents were alcoholic as well as the maternal grandparents. Inbred from mother by son. This animal was one of a litter of two stillborn. The left hind foot, C, had only one toe and the right, D, one toe and a stump, A and B, normal right and left hind feet.

opaque cornea, cataract or opaque lenses, small defective eyes, complete absence of one eye and finally complete absence of both eyeballs—anophthalmic monsters. In the latter case the extrinsic eye muscles, the third, fourth and sixth nerves, the lachrymal glands and other struc-

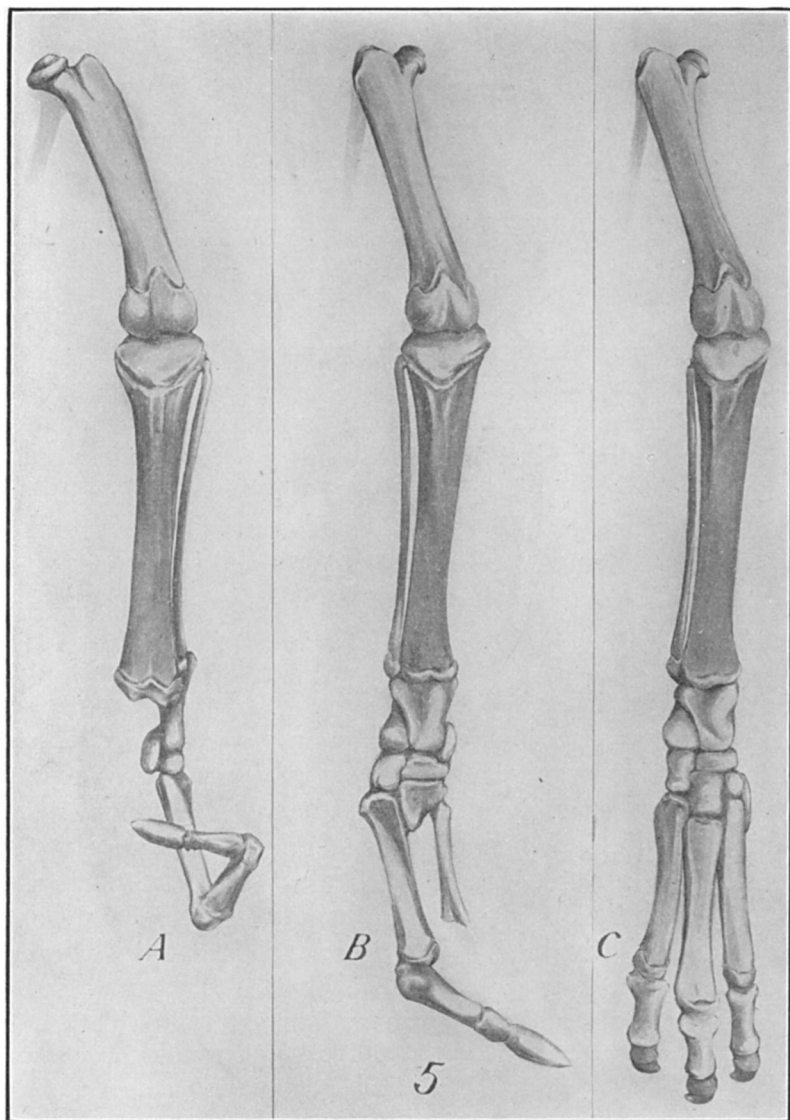


FIG. 5. The skeleton of the two limbs from Fig. 4 shows the left foot, *A*, to have only one metatarsal and toe, the third, and three tarsal bones. The right foot, *B*, has the third toe also and the first metatarsal with the tarsus almost complete. *C* shows the normal skeleton of a right hind limb with the three toes and seven tarsal bones.

tures of the orbit are present, though the eyeball is completely wanting.

Not only are the above congenital eye defects present, but in several instances members of the alcoholic lines have become blind during the first year or year and a half after birth, whereas in our control this has never occurred.

The several illustrations referred to above show specimens exhibiting these various defects. Figs. 1, 2 and 4 of Plates I and II are photographs of animals of indicated lineage which show paralytic conditions. Figs. 4 and 5 illustrate defective extremities. Figs. 1 to 3 show various degrees of defective eyes and absence of eyeball.

It is peculiarly interesting to find these particular eye conditions exhibited by the descendants of alcoholized animals, since, as Stockard ('10) has previously shown, closely similar eye conditions are obtained in great numbers by directly treating the eggs of fish with solutions of alcohol; and like conditions were also obtained, though not so consistently, by treating hens' eggs ('14) with alcohol fumes either before or during incubation.

The table just considered gives only a general idea of the experiment and is in no way analytical. We shall now attempt to analyze these data in such a manner as to determine the influence of internal factors, as, for example, inbreeding on the results. The influence of the size of the litter on the quality of the offspring. The behavior of  $F_1$  and  $F_2$  individuals derived from different lines, and whether there is a difference in the effects on male and female animals, and the manner of transmission of these effects.

*(To be continued.)*